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## **Abstract**

Long-term agricultural management may change soil C sequestration and alter soil C and N dynamics. The objective of this study was to investigate the impact of several tillage regimes with different intensity on C and N stocks in a Calcic Haploxeralf with a leguminous/cereal rotation under semiarid conditions after 15, 18 and 21 years of management. Seven chemical and biochemical properties (total C, total N,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , FDA hydrolysis,  $\beta$ -glucosidase and urease activities) were measured in a soil (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm) under the following agricultural management: fallow (F), no-tillage (NT), zone-tillage subsoiling with a paraplow (ZT), conventional tillage with mould board plow (CT), minimum tillage with chisel plow after NT (MTN) or CT (MTC). The results showed that soil reached a steady state of organic matter sequestration 15 years after starting the experiment and that C and N stocks varied greatly with agricultural management, particularly in the top 0-10 cm, and followed the order:  $F \approx NT \approx ZT > MTN \approx MTC > CT$ . Fallow and less intensively cultivated soils (NT, ZT) exhibited strong vertical gradients of most properties analyzed (total C, total N, FDA hydrolysis, urease and  $\beta$ -glucosidase activities) with values decreasing with depth, followed by minimum tillage treatments (MTN, MTC) whereas similar values along soil profile were observed in CT treatment. No significant differences in soil  $\delta^{13}\text{C}$  values were detected among plots with different land use or tillage systems; however, the  $\delta^{15}\text{N}$  values suggested that, although tillage system did not affect significantly N-cycling processes, a change from "open" to "closed" N cycling occurred when cultivated soils were set aside.

**Keywords:** C stock;  $\delta^{13}\text{C}$ ; fallow; N stock;  $\delta^{15}\text{N}$ ; soil enzymes; tillage systems

## **1. Introduction**

Soil physical, chemical and biological properties and processes are strongly influenced by soil organic matter (SOM) content, which is a key attribute in soil quality and productivity (Gregorich et al., 1994) and plays an important role in the global C budget through sequestration of atmospheric C (Lal, 2001). Assessment of SOM is therefore a valuable step to identify the overall soil quality and the sustainability of land management. In agricultural soils, conventional tillage may cause a substantial decrease of SOM content and labile pools of nutrients (Elliott, 1986; Karlen et al., 1994; Wander and Bollero, 1999). Conservation tillage minimizes soil disturbance and maintains crop residues on the soil surface, reducing their decomposition and leading to organic matter accumulation in the upper soil layer (Balesdent et al., 2000). Although the adoption of conservation practices may temporarily reduce plant available N through increased N immobilization (Doran, 1987),

conservation tillage improves N availability to plants in the long-term (Rice et al., 1986) by increasing soil N retention and labile N pool (Franzluebbers et al., 1994; McCarty and Meisinger, 1997) in the upper soil layers. Consequently, the change of tillage methods to reduced- or no-tillage practices is recommended to sequester organic C and hence to reduce the net emission of greenhouse gases (Lal, 2001).

Short- and medium-term variations in SOM following a change in soil management or land use are less well understood because they are difficult to measure by conventional methods. Stable isotopes measurements at natural abundance levels are a powerful research tool in environmental sciences (Handley and Scrimgeour, 1997; Robinson, 2001; Yakir and Sternberg, 2000). In the case of soils,  $\delta^{13}\text{C}$  has been usefully employed to monitor long-term intensive land use effects on SOM (Kalbitz et al., 2000) and  $\delta^{15}\text{N}$  values reflect the net effect of biotic and abiotic environment on

N-cycling processes (Dawson et al., 2002), being influenced by the quantity and quality of SOM inputs, N sources and isotopic fractionation during N transformations (Nadelhoffer and Fry, 1988). Likewise, the measurement of biochemical properties such as those related with mass and activity of soil microbial communities is recommended to detect SOM changes due to land-use and soil management over short and medium time scale (Madejon et al., 2007; Sparling, 1998). To this respect, studies of diverse authors have shown that the biochemical properties were more sensitive than total organic C and N for assessing the impact of different tillage practices on soil quality (Bergstrom et al., 1998; Biederbeck et al., 1994; Carter, 1986; Díaz-Raviña et al., 2005; Madejon et al., 2007; Madejon et al., 2009; Melero et al., 2012; Saffigna et al., 1989).

The aim of present work was to evaluate whether changes in SOM (including  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) and soil biochemical properties (FDA hydrolisis and b-glucosidase and urease activities) could be detected after 15-21 years under six management systems: conventional tillage (CT), minimum tillage after CT (MTC), minimum tillage after no tillage (MTN), no-tillage with paraplow (ZT), no tillage (NT) and fallow soil (F).

## 2. Material and methods

### 2.1. Site description and experimental design

The study was done in a long-term tillage experiment established at the CSIC Experimental Station (Toledo, Central Spain). The site is 450 m asl (Latitude 40°3', Longitude 4°26') on a loamy sand Calcic Haploxeralf (Soil Survey Staff, 2010). The area has a semiarid continental climate with (minimum and maximum average temperatures of 6 °C in winter and 23 °C in summer). The annual precipitation averages 428 mm, of which 28% in spring, 10% in summer, 26% in autumn and 36% in winter. The aridity index, i.e. the ratio of annual mean precipitation to annual mean evapo-transpiration, is 0.564 reflecting a semiarid climate which is typical of steppes and Mediterranean countries.

Two tillage systems were initially applied: conventional tillage with mouldboard plow (CT) and no-tillage (NT) in a randomised complete block design with nine replications (plots measured 9 m wide and 40 m long). After 7 years, three of the nine plots under NT were changed to minimum tillage with chisel plow (MTN) and other three to zone tillage with paraplowing (ZT) whereas three of the

nine plots under CT were changed to minimum tillage with chisel plow (MTC). Thus, the five tillage systems applied by triplicate were: NT, ZT, MTN, MTC and CT. The crop sequence was chickpea (*Cicer arietinum* L.) cv. Gracia/barley (*Hordeum vulgare* L.) cv. Volley, selected for their suitability in the climatic conditions of a dry farming experimental site. Cultural practices were similar to those employed by local farmers, adapted to the type of soil, weed incidence, etc., and remained constant for each crop and tillage system since the study began. CT consisted of fall ploughing to an average depth of 25–30 cm, followed by one or two passes with spring tine cultivator (10–15 cm depth) for seedbed preparation. Minimum tillage (MTN and MTC) involved chisel ploughing to an average depth of 15–20 cm. The ZT subsoiler (paraplow) was applied in alternate years and set to operate at a depth of 30 cm with little disturbance of the soil surface. Chemical fertilizers were applied in the same quantity for all treatments at barley pre-sowing in a mixed form (8–15–15 N–P–K) and as a top-dressing, at the tillering stage in the form of calcium ammonium nitrate (33% N), at an average total rate of 90–60–60 kg N–P–K ha<sup>-1</sup> (adjusted to supply the average uptake of the crops). Chickpea crop received at pre-sowing the same mixed fertilizer but at a lower rate (16–30–30 kg N–P–K ha<sup>-1</sup>). Crop yields (barley and chickpea) were harvested after reaching physiological maturity, usually in early July.

### 2.2. Sampling and analysis of soil

Soil was sampled after harvest of crops at different times after the establishment of experiment (15, 18 and 21 years). Eight soil sub-samples in each plot were taken at 0–5, 5–10, 10–20 and 20–30 cm depth using an auger. The sub-samples were mixed to produce a composite sample for each treatment, layer and plot. Additionally, a control soil (F) under shrub vegetation dominated by *Retama sphaerocarpa* (L) and other plants characteristics of semiarid ecosystems (*Silene vulgaris*, *Medicago minima*, etc.) was sampled at random in an adjacent (50 m apart) agricultural soil without human disturbance during the last 30–40 years, but in this case only a composite sample for each depth was taken. Chemical analyses were performed on soil samples collected at three sampling times (t= 15, 18 and 21 years after the experiment setup) whereas biochemical properties were only analyzed in samples collected at t= 15 years. After sieving at 2 mm, the homogenized soil samples were separated in two fractions, one was air-dried and used for measurements of chemical properties and the other was stored at 4 °C for no longer than 4 weeks until

analysis of biochemical properties.

Total C, total N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured on finely ground ( $< 100\ \mu\text{m}$ ) soil samples with an elemental analyser (Carlo Erba CNS 1508) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). The following rules, some of them also recommended by Jardine and Cunjak (2005), were taken into account in isotopic analysis. We constraint the weights of samples (analysed on duplicate) and standards such that their peaks' amplitudes were within a small range, and we adjusted to this range the peak of the internal reference injected in each analysis ( $\text{CO}_2$  or  $\text{N}_2$  from a pressure bottle calibrated against IAEA standards). With regard to C isotopic analysis, accuracy and precision for isotope reference materials IAEA-CH-6 and IAEA-CH-7 (included, alternately, after every tenth sample) were always within the certified values ( $-10.40 \pm 0.20\ ‰$  and  $-31.80 \pm 0.20\ ‰$ , respectively). The same was true for N with isotopic standards IAEA-N1 and IAEA-N2 ( $0.40 \pm 0.20\ ‰$  and  $20.3 \pm 0.20\ ‰$ , respectively).

The hydrolysis of fluorescein diacetate (FDA), an overall index of activity of heterotrophic microorganisms, and the measurement of two specific enzyme activities related with the C ( $\beta$ -glucosidase) and N (urease) cycles were used as indicators of soil microbial activity. Fluorescein diacetate (FDA) hydrolysis was determined as reported by Schnurer and Rosswall (1982) by incubating the soil samples with a solution of fluorescein diacetate for 1 h at  $24\ ^\circ\text{C}$ . The  $\beta$ -glucosidase activity was measured following the procedure of Eivazi and Tabatabai (1988), which determines the released *p*-nitrophenol after incubation of the soil samples with a 4-nitrophenyl- $\beta$ -D-glucopyranoside solution for 3 h at  $37\ ^\circ\text{C}$ . The urease activity was estimated by incubating the soil samples with an aqueous urea solution and extracting the  $\text{NH}_4^+$  produced with 1 M KCl and 0.01 M HCl followed by the colorimetric  $\text{NH}_4^+$  determination by a modified indophenol reaction (Kandeler and Gerber, 1988).

All analyses were carried out in duplicate and the mean of both analyses was used in the statistical procedures and were expressed on the basis of oven-dried ( $105\ ^\circ\text{C}$ ) weight of soil (absolute values).

### 2.3. Statistical analysis

Data on biochemical properties, measured only 15 years after the establishment of experiment, were statistically analyzed by two-way ANOVA to determine the percentage of variation attributable

to the factors tillage system (NT, ZT, MTN, MTC and CT) and soil depth. For the chemical properties, the exploratory analyses showed that soil total C, total N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  did not vary significantly among sampling dates (15, 18 and 21 years after the establishment of experiment); therefore, data of the three years were jointly analysed by two-way ANOVA (with treatment and soil depth as factors). The Levene's test was used for verifying the equality of variances among groups. In the case of homocedasticity, significant differences among the mean groups (F, NT, ZT, MTN, MTC and CT) were established at  $p < 0.05$  using the Bonferroni's test for multiple comparisons. In the case of unequal variances, the original data were subjected to the Tukey's ladder of power, or to Cox-Box transformations, to obtain equality of variances and then significant differences among the mean groups were established at  $p < 0.05$  using the Bonferroni's test. Statistical procedures were performed with SPSS 15.0 for Windows.

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## 3. Results

No significant differences were found for soil total C, total N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among the sampling years (15, 18 and 21 years after the experiment began). The two-way ANOVA showed significant and important effects of treatment, depth and their interaction on soil C (44%, 61% and 58% of variance explained, respectively; Table 1). The C content decreased with depth and was higher in F, NT and ZT than in CT, MTC and MTN treatments (Table 2; Fig. 1). No significant effect of treatment was found on soil  $\delta^{13}\text{C}$ , which increased significantly with depth (21% of variance explained; Tables 1 and 2).

Like for C, the two-way ANOVA showed significant effects of treatment, depth and their interaction on soil N (24%, 65% and 32% of variance explained, respectively; Table 1), N content decreasing with depth and being higher in F, NT and ZT than in CT, MTC and MTN treatments (Table 2; Fig. 2). Both treatment and depth have significant effects on soil  $\delta^{15}\text{N}$  (19% and 34% of variance explained; Table 1), whose values increased with depth and were lower in fallow than in all cultivation treatments (Table 2; Fig. 3).

Table 1. Results of the two-way ANOVA for the soil total C,  $\delta^{13}\text{C}$ , total N and  $\delta^{15}\text{N}$  with treatment (T) and depth (D) as factors. Note: ns, not significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; partial  $\eta^2$ , variance explained.

Variable	Treatment		Depth		Interaction (TxD)	
	partial $\eta^2$	p	partial $\eta^2$	p	partial $\eta^2$	p
Total C	440	***	0.609	***	581	***
$\delta^{13}\text{C}$	103	ns	215	***	239	***
Total N	235	***	647	***	321	***
$\delta^{15}\text{N}$	190	***	336	***	119	ns

Table 2. Soil total C,  $^{13}\text{C}$ , total N and  $^{15}\text{N}$  (mean  $\pm$  s.d) for the different soil management systems and soil depths. For each variable and factor (management system, soil depth), different letters show significant differences among groups ( $p < 0.05$ ). Treatments: F, fallow; NT, no tillage; ZT, zone-tillage subsoiling with paraplow; minimum tillage with chisel plow after no-tillage (MTN) or conventional tillage (MTC); conventional tillage with mouldboard plow (CT).

		Total C (g kg <sup>-1</sup> )	$\delta^{13}\text{C}$ (‰)	Total N (g kg <sup>-1</sup> )	$\delta^{15}\text{N}$ (‰)
Management system	CT	5.31 $\pm$ 0.62 <sup>b</sup>	-25.14 $\pm$ 0.42 <sup>a</sup>	0.547 $\pm$ 0.071 <sup>b</sup>	5.14 $\pm$ 0.94 <sup>a</sup>
	MTC	5.78 $\pm$ 1.19 <sup>b</sup>	-25.22 $\pm$ 0.33 <sup>a</sup>	0.598 $\pm$ 0.125 <sup>b</sup>	5.17 $\pm$ 0.88 <sup>a</sup>
	MTN	5.86 $\pm$ 1.22 <sup>b</sup>	-25.04 $\pm$ 0.74 <sup>a</sup>	0.549 $\pm$ 0.108 <sup>b</sup>	4.90 $\pm$ 0.75 <sup>a</sup>
	ZT	7.50 $\pm$ 3.12 <sup>a</sup>	-24.70 $\pm$ 1.19 <sup>a</sup>	0.712 $\pm$ 0.241 <sup>a</sup>	5.22 $\pm$ 0.99 <sup>a</sup>
	NT	7.95 $\pm$ 4.15 <sup>a</sup>	-24.93 $\pm$ 0.95 <sup>a</sup>	0.763 $\pm$ 0.336 <sup>a</sup>	5.29 $\pm$ 0.93 <sup>a</sup>
	F	8.85 $\pm$ 2.74 <sup>a</sup>	-24.79 $\pm$ 1.84 <sup>a</sup>	0.758 $\pm$ 0.359 <sup>a</sup>	3.94 $\pm$ 1.42 <sup>b</sup>
Soil depth	0-5 cm	9.32 $\pm$ 3.60 <sup>a</sup>	-25.43 $\pm$ 0.73 <sup>c</sup>	0.922 $\pm$ 0.297 <sup>a</sup>	4.37 $\pm$ 0.92 <sup>c</sup>
	5-10 cm	6.75 $\pm$ 1.36 <sup>b</sup>	-25.09 $\pm$ 0.68 <sup>b</sup>	0.680 $\pm$ 0.142 <sup>b</sup>	4.86 $\pm$ 0.68 <sup>b</sup>
	10-20 cm	5.44 $\pm$ 0.99 <sup>c</sup>	-24.83 $\pm$ 0.97 <sup>b</sup>	0.532 $\pm$ 0.066 <sup>b</sup>	5.13 $\pm$ 0.96 <sup>b</sup>
	20-30 cm	4.80 $\pm$ 1.20 <sup>d</sup>	-24.57 $\pm$ 0.98 <sup>a</sup>	0.478 $\pm$ 0.080 <sup>c</sup>	5.78 $\pm$ 0.97 <sup>a</sup>

The biochemical properties values obtained for soil samples studied are shown in Fig. 4. In the uncultivated soil (F) the FDA hydrolysis ranged from 10.8 to 24.3  $\mu\text{g}$  fluorescein  $\text{g}^{-1} \text{h}^{-1}$  ( $14.7 \pm 3.2 \mu\text{g}$  fluorescein  $\text{g}^{-1} \text{h}^{-1}$ , mean value  $\pm$  SE), the glucosidase activity varied from 43 to 216  $\mu\text{g}$  p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  ( $111 \pm 38 \mu\text{g}$  p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ) and the urease activity ranked from 10.7 to 37.2  $\mu\text{g}$   $\text{NH}_4^+$   $\text{g}^{-1} \text{h}^{-1}$  ( $20.1 \pm 5.8 \mu\text{g}$   $\text{NH}_4^+$   $\text{g}^{-1} \text{h}^{-1}$ ). In the cultivated soils the ranges of values were 11.4-23.2  $\mu\text{g}$  fluorescein  $\text{g}^{-1} \text{h}^{-1}$  ( $17.2 \pm 0.9 \mu\text{g}$  fluorescein  $\text{g}^{-1} \text{h}^{-1}$ , mean values  $\pm$  SE) for FDA hydrolysis, 70-247  $\mu\text{g}$  p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  ( $128 \pm 11 \mu\text{g}$  p-nitrophenol  $\text{g}^{-1} \text{h}^{-1}$ ) for glucosidase and 8.7-45.4  $\mu\text{g}$   $\text{NH}_4^+$   $\text{g}^{-1} \text{h}^{-1}$  ( $20.2 \pm 2.5 \mu\text{g}$   $\text{NH}_4^+$   $\text{g}^{-1} \text{h}^{-1}$ ) for urease. A positive and significant ( $p < 0.001$ ,  $n$

= 24) relationship between properties related to organic matter content and  $\beta$ -glucosidase ( $r = 0.816$  for total C and  $r = 0.769$  for total N) and urease activity ( $r = 0.746$ - $0.747$  for total C and N) was observed. Likewise, a significantly positive relationship between  $\beta$ -glucosidase and urease activities was observed ( $r = 0.85$ ,  $p < 0.001$ ,  $n = 24$ ), being the  $\beta$ -glucosidase activity also related to FDA hydrolysis ( $r = 0.68$ ,  $p < 0.001$ ,  $n = 24$ ).

The two-way ANOVA showed that depth and tillage systems (16-67% and 11-37% of variance explained, respectively) have a significant effect on the biochemical properties of cultivated soils expressed as absolute values. To compare the variation of biochemical properties along the soil

profile, stratification ratios were calculated from values at 0-5 cm divided by those at 20-30 cm. The stratification values of FDA hydrolysis varied following the order F (2.2) > NT, ZT and MTN (1.5-1.8) > MTC and CT (0.9-1.1). The stratification ratio of  $\beta$ -glucosidase activity showed a similar pattern being greater under uncultivated soil (value of 5) than in the corresponding cultivated soils (3.1-1.5) and decreasing gradually with the tillage intensity showing values of 2.9-3.1, 2.2-2.1 and 1.5 under no tillage (NT and ZT), minimum tillage (MTN and

MTC) and conventional tillage (CT), respectively. In contrast, the stratification ratio of urease activity values was greater under MTC and CT compared with the rest of treatments (F, NT, ZT and MTN). When biochemical properties were expressed as percentage of total C (relative values, data not shown), the influence of depth as a source of variation decreased, explaining only 10-16% of variance, whereas the importance of tillage system increased notably (15-48% of variation).

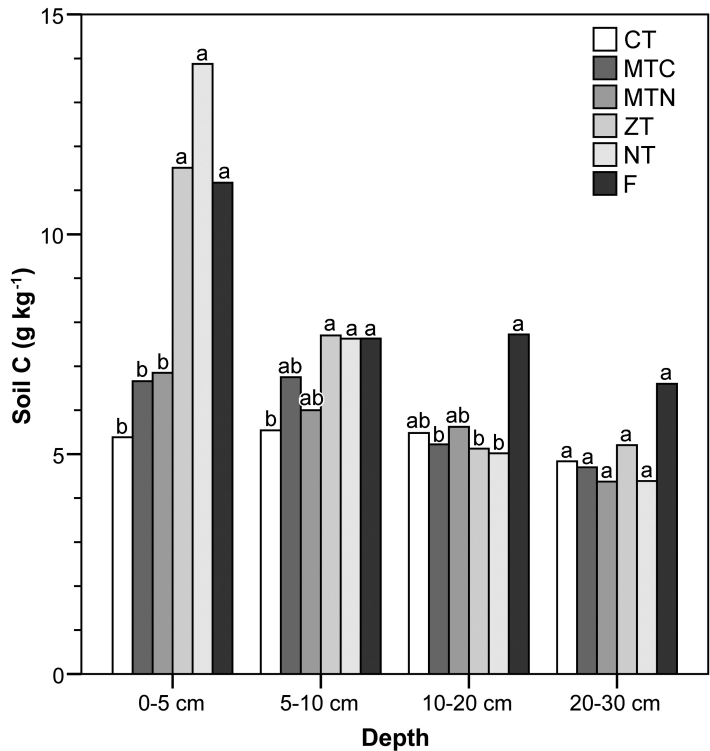


Fig. 1. Soil total C for the different soil management systems and soil depths. For each soil depth, different letters show significant differences among soil management systems ( $p < 0.05$ ). Treatments: F, fallow; NT, no tillage; ZT, zone-tillage subsoiling with paraplow; minimum tillage with chisel plow after no-tillage (MTN) or conventional tillage (MTC); conventional tillage with mouldboard plow (CT).

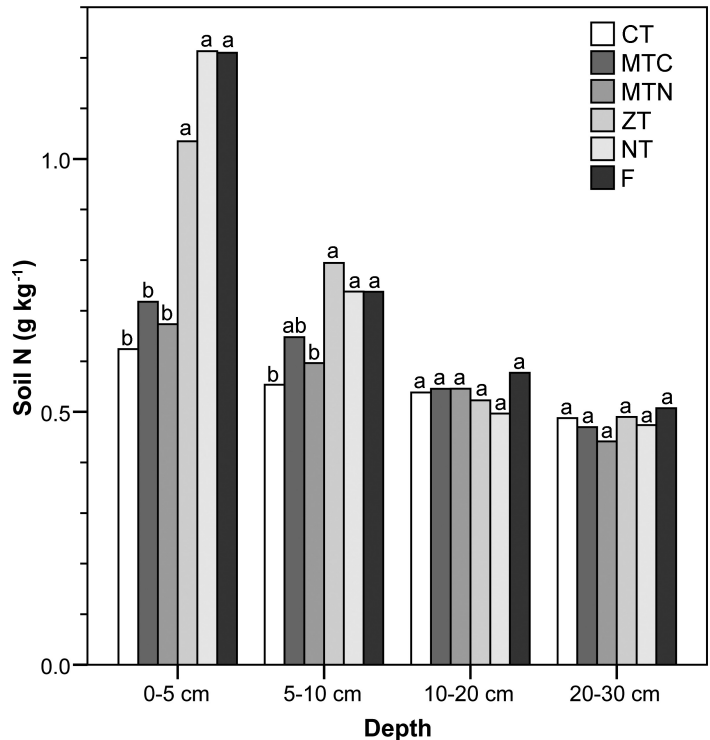


Fig. 2. Soil total N for the different soil management systems and soil depths. For each soil depth, different letters show significant differences among soil management systems ( $p < 0.05$ ). Treatments: F, fallow; NT, no tillage; ZT, zone-tillage subsoiling with paraplow; minimum tillage with chisel plow after no-tillage (MTN) or conventional tillage (MTC); conventional tillage with mouldboard plow (CT).

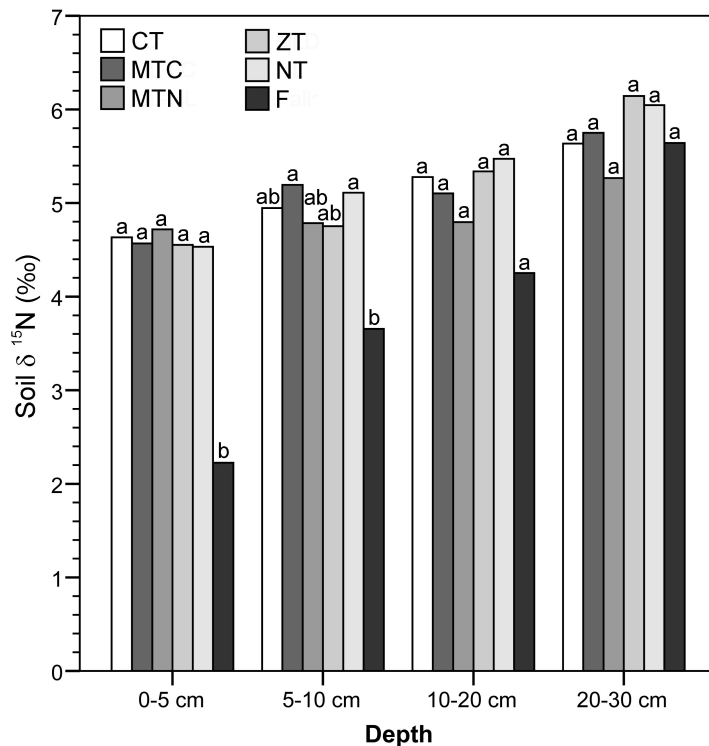


Fig. 3.

Soil total  $^{15}\text{N}$  for the different soil management systems and soil depths. For each soil depth, different letters show significant differences soil management tillage systems ( $p < 0.05$ ). Treatments: F, fallow; NT, no tillage; ZT, zone-tillage subsoiling with paraplow; minimum tillage with chisel plow after no-tillage (MTN) or conventional tillage (MTC); conventional tillage with mouldboard plow (CT).

#### 4. Discussion

The lack of significant differences for soil total C, total N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among the sampling years (15, 18 and 21 years after the experiment began) suggests that, irrespectively of the management system (NT, ZT, MTN, MTC and CT), the SOM inputs and outputs have reached an equilibrium in the studied soils, with higher SOM contents in fallow and no tillage plots than in those under conventional or minimum tillage. According with this result, no more SOM accumulation must be expected in these semi-arid soils after 15 years of no tillage. Hernanz et al. (2009) in a cereal/leguminous rotation with three tillage systems (NT, MT, CT) in central Spain have also reported that steady state of organic matter sequestration in a Vertic Luvisol was reached after 11 years of starting the experiment in NT and 12 years in CT and MT. For the SOM variables, results also showed that differences among tillage systems decreased with depth and are not significant in the 10-20 and 20-30 cm soil layers. Although further studies should be focussed on a short-term scale ( $< 15$  years) in order to determine more precisely when the equilibrium is reached, results clearly indicate that in this Calcic Haploxeralf from central Spain under semiarid climatic conditions SOM changes occurred rapidly.

Compared with fallow plots, the soil C content was reduced by 40% under conventional tillage and by 35% under minimum tillage, with no significant differences among these tillage systems.

Conversely, with a reduction in soil C of only 10-15%, the no tillage treatments were statistically grouped with fallow plots and clearly differentiated from conventional and minimum tillage treatments. Similarly, other studies have shown the effectiveness of reduced- and no-tillage systems for C sequestration and hence for mitigating climate change (Díaz-Raviña et al., 2005; Dick et al., 1998; Follett, 2001; Hernanz et al., 2009; Lal, 2001; Lopez-Fando and Pardo, 2009). It should be noticed, however, that soil potential for C sequestration is finite and limited by soil depth (marked effects only in the first 0-10 cm). Moreover, specific environment conditions (soil type, farming system and climate) are also determinant in C sequestration because the SOM content at equilibrium depends on the interaction of factors as OM inputs, rates of endogenous SOM and exogenous OM mineralization, soil texture and climate (Johnston et al., 2009). These facts should be taken into account when the effects of conservation tillage on C cycle in soils under different environments were evaluated (e.g. semiarid and temperate humid conditions); however, the aspects related to the importance of the SOM level at equilibrium, including the time needed to reach it and the soil productivity when attained, are often ignored in published investigations, even those concerning long-term sustainability of agricultural systems.

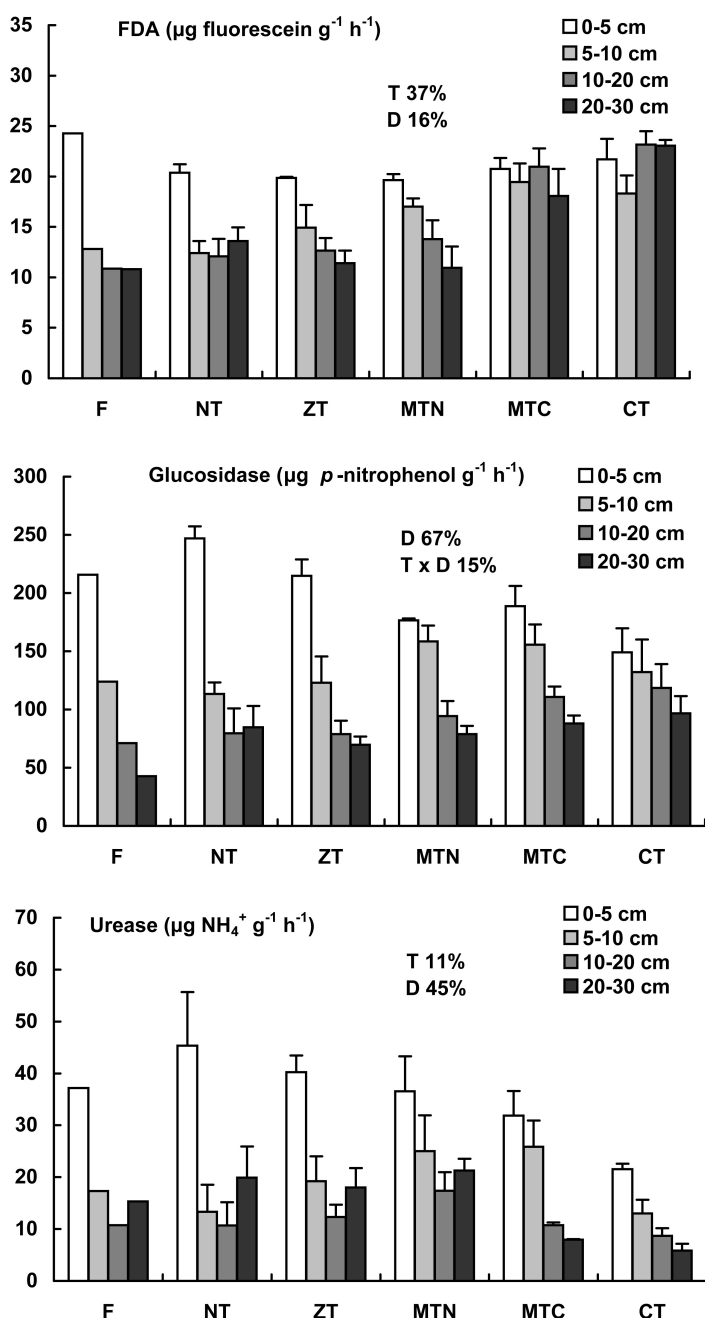


Fig. 4.

Biochemical properties measured at four depth increments in the different soil management systems (mean  $\pm$  s.d.;  $n=3$  field replicates). For each analyzed parameter ANOVA 2 (T, tillage system; D, depth; T $\times$ D, tillage system $\times$ depth interaction) were performed, but only proportion of variance explained by significant factors ( $p < 0.05$  level) are indicated. Treatments: F, fallow; NT, no tillage; ZT, zone-tillage subsoiling with paraplow; minimum tillage with chisel plow after no-tillage (MTN) or conventional tillage (MTC); conventional tillage with mouldboard plow (CT).

Soil organic matter  $\delta^{13}\text{C}$  is affected by the isotopic signature of vegetation inputs, fractionation during microbial decomposition and soil characteristics (mineralogy, clay content and pH) (Dijkstra et al., 2006; Ehleringer et al., 2000; Krull and Skjemstad, 2003). Despite a slight tendency to more negative  $\delta^{13}\text{C}$  values as tillage intensity increase, no significant differences were found among treatments. Soil  $\delta^{13}\text{C}$  became progressively less negative with soil depth, with a significant difference of 0.9 ‰ between the most superficial and the deepest soil layer and intermediate values in the 5-10 and 10-20 cm layers. This trend agrees with the typical increase in  $\delta^{13}\text{C}$  by 1-3 ‰ in subsurface horizons (Ehleringer et al.,

2000), although a decrease has also been reported in Vertisols. Changes in SOM  $\delta^{13}\text{C}$  below 1 ‰ must be interpreted cautiously (Marriott et al., 1997), but the enrichment with depth we found can be due to: (a) the 1.5 ‰ decrease of  $\delta^{13}\text{C}$  in atmospheric  $\text{CO}_2$  since industrialization (Francey et al., 1999); (b) isotopic fractionation during microbial decomposition and addition of  $^{13}\text{C}$ -enriched microbial biomass (Dijkstra et al., 2006; Heil et al., 2000; Krull and Skjemstad, 2003); and/or (c) a gradual shift in the relative contributions of microbial vs. plant components in the residual SOM (Ehleringer et al., 2000).

Taking the fallow plots as reference, the decrease of soil N content due to tillage was lower than that

found for C: 28%, 11-18% and 0-6% under conventional tillage, minimum tillage and no tillage, respectively. These results agree with those of Díaz-Raviña et al. (2005) who reported that conservation tillage mitigates the negative environmental impacts of conventional tillage by reducing N losses.

Díaz-Raviña et al. (2005) and Gómez-Rey et al. (2012) found higher soil  $\delta^{15}\text{N}$  under plough tillage than under conservation tillage in a Phaeozem Gleyic (IUSS Working Group, 2006) from the Spanish temperate humid region. However, not all environmental conditions may manifest this potential change. Thus, contrastingly, in present study [Calcic Haploxeralf (Soil Survey Staff, 2010) from a semi-arid region] all tillage plots have mean soil  $\delta^{15}\text{N}$  values within a tight range (4.90 to 5.29 ‰), clearly differentiated from those of fallow plots (3.94 ‰). This result cannot be related with the fertilization of the cultivated plots because most N fertilizers are synthesized from the atmospheric  $\text{N}_2$  and their isotopic signatures were around 0 ‰ in Spain and elsewhere (Vitoria et al., 2004). Therefore, the higher  $\delta^{15}\text{N}$  values in the cultivated soils are likely related with increased N outputs (nitrate leaching, ammonia or N oxides volatilization) that discriminate against the heavy isotope and, consequently, are  $^{15}\text{N}$  depleted (Högberg et al., 1995); in the same way, Abadín et al. (2002) also found a reduction in soil  $\delta^{15}\text{N}$  with fallow age. The usefulness of  $\delta^{15}\text{N}$  as potential tracer of changing land use is clearly showed since  $\delta^{15}\text{N}$  values allow us to discriminate among soils with similar C and N stocks but under differing land use (eg. F treatment from NT and ZT treatments). Studies should be performed with a wide range of agricultural soils under different environments (soil type, farming system, climate) in order to confirm these previous results concerning the organic matter dynamics following soil management as well as the usefulness of incorporating  $\delta^{15}\text{N}$  measurements in such investigations. The similar  $\delta^{15}\text{N}$  values among different cultivated soils (NT, ZT, MTN, MTC, CT) suggest that, under these circumstances (soil type, fertilizer management, semiarid climate, low input of organic material, low crop production), N-cycling processes are not significantly affected by tillage system. An increase in soil  $\delta^{15}\text{N}$  with depth, as we found in present study, is the most usual trend in soils (especially in forests) and has been related with the redeposition of  $^{15}\text{N}$ -depleted plant N onto the soil surface, the discrimination of  $^{15}\text{N}$  by microbial decomposition and soil characteristics (Heil et al., 2000; Högberg, 1997; Krull and Skjemstad, 2003). As expected, the  $^{15}\text{N}$  enrichment

with depth was much more important in fallow plots than in the ploughed ones (Fig. 3) because above-ground phytomass is taken away at harvests or mixed into deeper soil layers by ploughing.

The enzymatic activity values, more influenced by soil depth (16-67% of variance) than by tillage system (11-37% of variance), were lower than those obtained for agricultural soils from temperate humid zone of NW Spain (Díaz-Raviña et al., 2005; Mahía and Díaz-Raviña, 2007; Mahía et al., 2011) but lied in the reported range for other Spanish soils in the semiarid and arid zones (Madejon et al., 2007; Madejon et al., 2009; Melero et al., 2012). Higher values were exhibited by the 0-5 cm layer of soil samples with higher organic matter content, which seems to indicate that the main soil property determining biochemical properties levels is the organic matter content. The positive correlations of total C and total N with FDA hydrolysis, -glucosidase and urease activities seem to support this assumption. This agrees with the finding of several authors indicating that microorganisms in most soil ecosystems are resource-limited (metabolisable C, available nutrients) (Díaz-Raviña et al., 1988; Wardle, 1992). Since SOM tended to decrease with soil depth and, in addition, soil management alter the SOM distribution along soil profile (see Fig. 1), different stratification ratios of soil biochemical properties were observed depending on agricultural management. In general, soils under fallow (F) showed the highest stratification ratios, the soils under conservation tillage systems (NT, ZT and MTN) intermediate values and finally the soils under the more intensive tillage systems exhibited the lower stratification ratios (MTC, LC). Greater stratification of C and N pools (either total or biologically active, i.e., soil microbial biomass and potential activity) with the adoption of conservation tillage systems was also observed by Franzluebbers (2002). These results suggest that the SOM stratification with depth, expressed as a ratio, could indicate soil quality or soil ecosystems functioning, because surface SOM is essential to erosion control, water infiltration and conservation of nutrients. Our results seem to support this assumption concerning the use of stratification of soil enzymes with depth as good indicators of dynamic of soil quality following agricultural management. A significantly positive relationship between -glucosidase activity with both FDA hydrolysis and urease activity was observed, emphasizing the interdependence of the activity of biogeochemical cycles of C (-glucosidase) and N (urease) and indicating that, in principle, they can respond similarly to the soil



management. However, the stratification ratios of these parameters seem to indicate that  $\alpha$ -glucosidase is more sensitive than FDA hydrolysis and urease for assessing the impact of agricultural management of these semiarid zone soils. This is consistent with findings of Melero et al. (2012) who, analyzing a wide range of soil properties (total C, total N, active carbon, water soluble carbon, dehydrogenase activity and  $\beta$ -glucosidase activity), also found that stratification of  $\beta$ -glucosidase was the best indicator of soil quality under different soil management in Mediterranean conditions.

## 5. Conclusions

Our results indicated that, compared with soil under fallow, conventional tillage led to a clear reduction in C and N stocks in the first 0-10 cm but no appreciable changes were detected below this depth. The adoption of minimum tillage (MTC, MTN) and no tillage practices (NT, ZT), particularly the later, led to an enhancement in total C and total N concentrations in the surface layer and, therefore, to a stratification of organic matter and enzymatic activity levels with depth. The data also showed that changes induced in SOM by agricultural management occurred rapidly in semiarid conditions since stocked SOM reached steady state after 15 years and then remained unchanged during the following 6 years period. In the present study the soil  $\delta^{15}\text{N}$  values suggested that tillage system had a limited effect on N cycling and that the abandonment of cultivated soils provoked a change from "open" to "close" N cycling. The usefulness of  $\delta^{15}\text{N}$  as potential tracer of changing land use is clearly showed since  $\delta^{15}\text{N}$  values allow us to discriminate among soils with similar C and N stocks but under differing land use (eg. F treatment from NT and ZT treatments).

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